Effect of salt on the micellisation of bile salts in aqueous medium

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CERTIFICATE

This is to certify that the dissertation entitled **"Effect of salt on the micellisation of bile salts in aqueous medium"** being submitted by Miss Swati Rekha Gouda to the Department of Chemistry, National Institute of Technology, Rourkela, Orissa, for the award of the degree of Master of Science is a record of bonafide research carried out by her under my supervision and guidance. To the best of my knowledge, the matter embodied in the dissertation has not been submitted to any other University / Institute for the award of any Degree or Diploma.

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Swati Rekha Gouda

CONTENTS

CHAPTER - 1

INTRODUCTION

1.1 BILE SALT:

Bile is a secreatory and excretory fluid found in vertebrate, including human. Bile salts are found in bile which are biosynthesized in liver by the cytochrome P_{450} mediated oxidation of cholesterol, is stored in gall bladder [1]. The daily secretion of bile in a human adult is 0.4 -0.8 L. The constituents of bile include lipids, bilirubin, and various inorganic and organic compounds [2]. Information about the constitution of bile was given years ago by Berzelius, Freiherrn von Gorup-Besanez [3] and Menzies [4]. The major biosynthesized bile acids are classified into cholic acid (CA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA), and ursodeoxycholic acid (UDCA). The quantitative ratio is 12 (CA):7 (DCA):13 (CDCA):1 (UDCA) in human gallbladder. [5] Bile acid is the protonated (-COOH) form of compound and bile salt is the deprotonated ($-COO^-$) form of compound [4]. Bile salts exist in solution as (1) an insoluble protonated acid (2) a dissolved protonated acid or its anions (3) micelles (4) a constituent of mixed micelles or vesciles (5) a soluble and a insoluble salts [6]. The function of bile is solubilisation of cholesterol, lipids, fatty acids, monoglycerides and fat soluble vitamins. This helps in effective digesion and absorption of lipids and fats. Thus it can be considered as a biosurfactants. Although every day large quantities of bile salts are secreted into the intestine, only moderate amounts are lost from the human body, because approximately 95 % of the bile salts delivered to the duodenum are reabsorbed into blood within the ileum. Based on this so-called enterohepatic circulation mechanism each bile salt molecule is “reused” about 20 times [7]. In the physiological environment they play a vital role in digesion and gall stone formation.

They are typical micelles which have characteristic structure and aggregation properties. This interesting physiological function of the molecule has made it a subject of extensive study.

Table 1.1: Concentration of bile salt in different body compartment of humans[8,9]

Compartment	Concentration
Gall bladder	10 – 50 mmol/L
Gut	4 – 50 mmol/L
Liver Canaliculi	5 mmol/L
Portal Vein Blood	0.1 mmol/L
Peripheral blood	5 - 12µmol/L

1.2 STRUCTURE:

Bile salt molecule composed of a steroid structure. The steroid structure has four rings and a five or eight carbon side-chain terminating in a carboxylic acid. . The four rings as labeled in Figure1.1 are (from left to right) A, B, C, and D, with the D-ring being smaller by one carbon than the other three. The hydroxyl groups are present in C3, C7 and C12 position in either alpha (α) or beta (β) orientation (Figure 1.2).

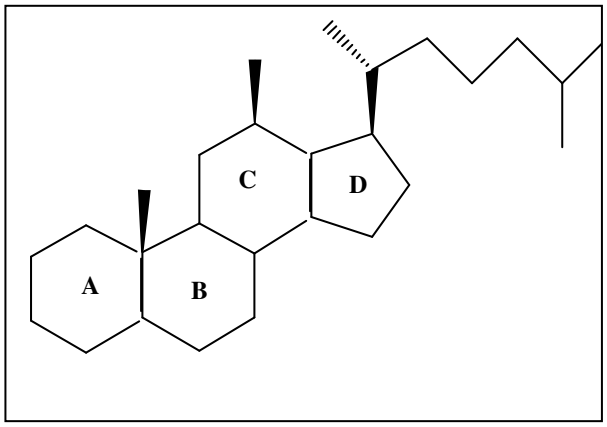


Fig. 1.1 Sterol ring of bile

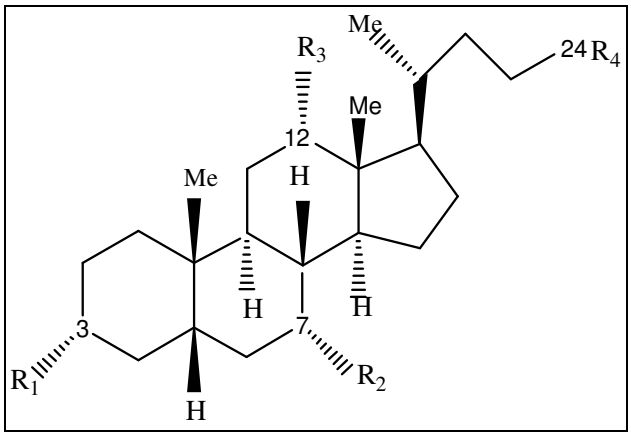


Fig. 1.2 Bile salt molecule with hydroxyl groups (R1,R2,R3) and COOH group (R4)

Table 1.2: Position and orientation of hydroxyl groups in a typical bile salt.[10]

Bile salt	**R1**	**R2**	**R3**
Cholate	αOH	αOH	αOH
Deoxycholate	αOH	αOH	H
Hydroxycholate	αOH	H	H

All bile acids have a hydroxyl group at position 3, which was derived from the parent molecule, cholesterol. In cholesterol, the 4 steroid rings are flat and the position of the 3-hydroxyl is beta (β). In many species, the initial step in the formation of a bile acid is the addition of a 7-alpha(α) hydroxyl group. Subsequently, in the conversion from cholesterol to a bile acid, the junction between the first two steroid rings (A and B) is altered, making the molecule bent, as a result of which, the 3-hydroxyl is converted to the alpha(α) orientation. Thus, the simplest bile acid (of 24 carbons) has two hydroxyl groups at positions 3α and 7α. Human bile salts differ on the basis of number, position and stereochemistry of hydroxyl group in 3, 7 ans 12 position of the steroid

ring (Figure 1.3). The presence of taurin or glycine conjugation in the bile salt also contributes to the variety in human bile salts [10,11].

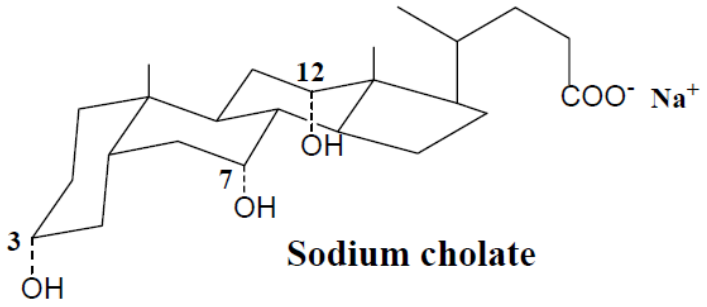


Fig. 1.3 Molecular structure of sodium cholate.

1. 3 MICELLISATION:

The chemical structure of bile salts is quite different from the classical amphiphilic molecule which has distinct hydrophilic head and hydrophobic tail (e.g-octyl glucoside or sodium dodecyl sulphate) [12,13]. Rigidity in the structure of bile salt results from the rigid steroid nucleus. The stereochemical arrangement of the two rings A and B (cis to each other) provides a kidney bean shape to the rigid frame work. It has a hydrophobic surface which is the convex side of the steroid ring system and a polar surface constituted of all hydroxyl groups present on the concave side of the system. Thus bile salt acquires a planar polarity gradient having hydrophobic domain on one side and hydrophilic domain on the other side. The planar polarity gives the molecules amphiphilic property and they tend to self organize when dispersed in an aqueous medium. The driving force for the aggregation property is the hydrophobic effect [12]. Similar to classical amphiphiles bile salts form micelles above a certain concentration called critical micellar concentration (CMC). The bile salt micelles formed are generally smaller than that of classical

micelles due to the planar structure of molecule which has to isolate the hydrophobic part of the molecule from aqueous phase. Determination of micellar structure on atomic level is difficult due to the dynamic nature of micelles. One- and two-dimensional NMR combined with molecular mechanics calculations indicate that the aggregates are stabilized by hydrophobic interactions and reduced electrostatic repulsion between the charged acidic groups [15,16]. However, the structure of a bile salt micelle have not been determined exactly [16] and the results are still under debate. Numerous models for micellar structure is proposed since last five decades.

1.3.1. Small's model

It is a two step model where the formation of primary micelles is followed by secondary micelles. Primary micelles consist of two to nine monomers and they are held together by hydrophobic interaction between the steroid nuclei. The primary micelles further aggregate and give rise to large aggregates, held together by hydrogen bonding between the hydroxyl groups of the primary micelles (Figure 1.4). The primary micelles are suggested to be globular in shape and the secondary micelles are roughly globular and have oblate ellipsoidal structure. The primary–secondary micelle scenario has been confirmed by simulation [17]. Though there still remains ambiguity this model however has gained more popularity among the researchers.

1.3.2. Oakenfull and Fisher's Model*:*

Oakenfull and Fisher suggested that the first stage of aggregation is due to dimer formation which includes maximum number of hydroxyl and charged carboxylic groups. The back-to-back hydrophobic interaction between the dimers helps formation of layer aggregates of bile salts (Figure 1.5). This -to-back hydrophobic interaction between the dimers is in synchronization

with Small's model for formation of primary micelles. The stacked micelles give a rod like structure to the secondary aggregation [15].

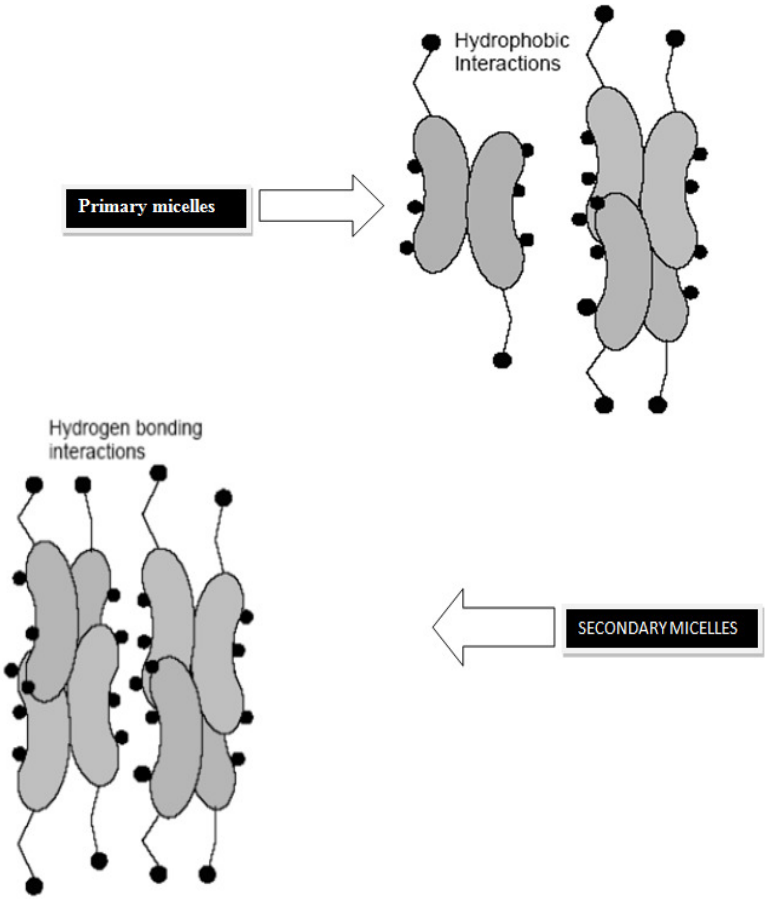


Fig. 1.4 Pictorial representation of bile salts micelles as suggested by Small [10]

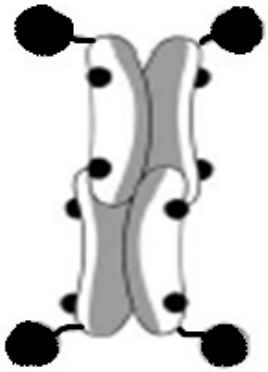


Fig 1.5. Pictorial representation of bile salts micelles as suggested by Oakenfull and Fisher [10]

1.3.3 Kawamura *et al.* Model:

The secondary micelle formation model was extended to the disc like model. Bile salt molecules are expected to associate with their hydrophobic faces oriented toward the interior of the micelle and the hydrophilic faces towards water, as a result of whic the hydrophobic faces are shielded from water (Figure 1.6). The long axes of the molecules are almost parallel, with an alternating orientation. This reduces electrostatic interactions and enhances charge density on the micellar surface which is lower than the spherical micelles, explaining the low counter ion binding. This model allows for a continuous increase of the micellar size. It can be applied to micelles of trihydroxy bile salts and dihydroxy bile salts where former has low aggregation numbers, and thus a rather loose structure, and later has bile salts with a more tense arrangement [19].

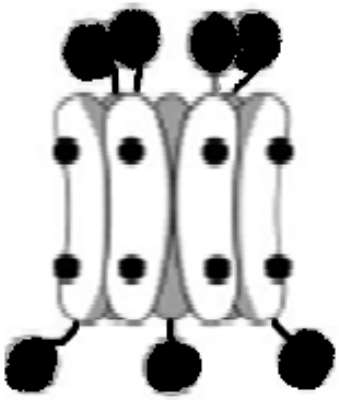


Fig 1.6. Pictorial representation of bile salts micelles as suggested by Kawamura et al. [10]

1.3.4 Warren *et al.* Model:

On the basis of structure of the crystalline state rather than the liquid state an alternative model of micelles was proposed which has helical shape. The aggregation is developed by polar interaction. The helix is filled with cations surrounded by water molecules, as in the case of

inverted micelles (Figure 1.7). However, on analysing the spontaneous bile salt aggregation based on molecular dynamic simulations, have discounted the "helix-model. Thus presently the disc shaped structure is widely accepted. [20]

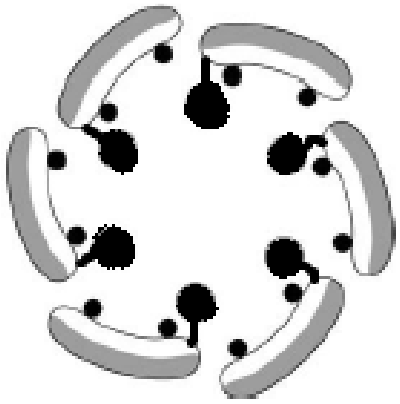


Fig 1.7. Pictorial representation of bile salts micelles as suggested by Warren et al. [10]

1.4 TECHNIQUES USED TO STUDY MICELLISATION PROCESS:

Numerous techniques have been adopted to study the process of micellisation. Some of them are described as follows:

Light Scattering: There are two light scattering experimental techniques:

Static Scattering: It is useful to find both the molecular weight and the aggregation number. In this method the excess scattering intensity is studied as a function of concentration of the bile salt [21,22].

Dynamic Scattering: This method is employed to determine the translational diffusion co-efficent and the hydrodynamic radius of micelles by quashi-elastic light scattering. It is sensitive and flexible compared to static scattering technique. The study of the change in the hydrodynamic radius as a function of concentration and temperature gives valuable information

on change in micellization equilibrium as well as change of the size and shape of the associated micelle involved [23,24].

Micro-Calorimetry: The volumetric specific heat is measured using a differential flow micro-calorimeter system. It measures density using a flow densitometer with vibrating tube working continuously [25].

Small Angle X-ray Scattering: This method is used to study inter-micellar ordering of semi dilute solution of micelles and the study of inter-micellar interaction. The principle of the technique is based on the electron density difference between solvens and the different parts of the soluble species, individual dimensions of the micellar core and those of the corona can be determined [25].

Transmission Electron Microscopy : It is employed to investigate micellar internal structure. In this technique a drop of dilute solution of micelle is spread on a carbon film and the solvent is evaporated. Then the dry isolated micelle can be stained and observed under microscope. In the recent years methods called cryo-TEM is used. The solution in this technique, is rapidly frozen by liquid nitrogen, stained and observed under appropriate low temperature condition [25]

Ultracentrifugation: Sedimentation velocity analysis of micellar solution can give valuable information on the size and distribution of micelles. In this method the velocity at which each solute species is displaced due to the influence of strong centrifugal force is measured [25].

Spectroscopic Techniques: This is the most effective and widely used tool to study various micellar properties:

Nuclear Magnetic Resonance (NMR): H^1 NMR spectroscopy has been used to study the solution phase of bile salts [26]. The study of relaxation time as a function of concentration gives us an idea about hydrophobic interaction between bile salt molecule in aqueous medium. C^{13} NMR gives the same information in addition to the molecular structure information on the aggregates.[27]

UV-Visible Spectroscopy: For this study generally a suitable chromophore is chosen so that the probe shows a change in any of its spectral properties such as optical density, energy maximum etc. when it is in the aqueous medium and in the micellar environment. The change in that property of the chromophore is then studied as a function of concentration of the surfactant, which gives us the value of critical micellar concentration (CMC) [28].

Fluorescence Technique: This technique is advantageous due to 1) high sensitivity and selectivity 2) minimum time consumption and 3) non-invasive nature. It is used to measure change in different fluorescence parameters of a suitable fluorescent probe. This change when studied as a function of various molecular properties like polarity, viscosity etc. provides immense information about the micro environment of the micelles[,29].

1.5. APPLICATION OF BILE SALT MICELLES:

Taminen and Kolekmainen [30] discovered the potential of bile acids as building blocks for the formation of supramolecular structures. These structures have got peculiar molecular recognition properties (example- bile acid-porphyrin conjugates are investigated with respect to their saccharide binding capacities) [31-33]. In the field of protein biochemistry bile salts are used to remove membrane bound proteins and other structures [34]. In the field of pharmaceutical sciences, bile salt micelles have been studied extensively because of their ability as drug carrier

systems [32,33,36]. The prime factor that makes a bile salt micelle a good vehicle for drug delivery is its ability to solubilise hydrophobic drugs. Various therapeutic drugs are commercialised as formulations containing bile salts, phospholipids and/or fatty acids. A commercial product with the trade name Valium MM [36,] for *I.V.* application contains the tranquilizer diazepam, a 1,4-benzodiazepin derivative, "solubilised" in mixed micelles (MM). The formulation contains glycocholic acid and soy lecithin as well as benzyl alcohol (preservative), sodium bisulfite, sodium chloride (HCl and NaOH) and water for injection. The bile salt loaded drug is found in a powder form, which is needed to be mixed with saline or distilled water so as to inject into the body. Human body is rich in salts of various cations and anions. Table 1.3 provides the list of cations and anions found in different body fluids.

Table 1.3. List of various cations and anions found in different body fluids

Electrolyte	Plasma (mEq/L) [Molarity]	Plasma Water (mEq/L) [Molality]	Intestinal Fluid (mEq/L)	Intracellular Fluid (mEq/L)
Cations				
Sodium	142	153	145	10
Potassium	4	4.3	4	160
Calcium	5	5.4	5	2
Magnesium	2	2.2	2	26
Anions				
Chloride	101	108.5	114	3
Bicarbonate	27	29	31	10
Phosphate	2	2.2	2	100
Sulphate	1	1	1	
Organic acid	6	6.5	7	
Protein	16	17	1	65

1.6. EFFECT OF SALT ON MICELLISATION:

The increase in ionic strength of the aqueous medium is known to promote the micellar aggregates of bile salts. The effect of counter-ion on the CMC of bile salt micelles is mainly

mediated by progressive neutralisation of the ionic charges on the carboxylate group. As the CMC is an index of hydrophobic interaction and ionic repulsion, reduction of the latter allows micelle formation to occur at lower concentration thus resulting in a decrease in the CMC (Reis *et al.*, 2004). The literature survey has brought out numerous explanations about the effect of salt on the micellisation process. Counter ions reduces the CMC in ionic and non ionic detergents by various mechanisms [37]. The major effect on the CMC due to added electrolyte is charge nutralisation but salting out effect concept proposed by Mukerjee [38] is quite considerable. The dependence of CMC and ion concentration can be proved thermodynamically. In order to prove it has been assumed that above CMC bile salts exist as monodispersed micelles and momomers in equilibrium [39]. The law of mass action is applied taking this assumption into consideration [40]. Let the concentration of bile salt be denoted as A^- , number of bile salt anions as "n" , counterion (say Na^+), number of counter ion bind to micelle is "m" and micelle itself is denoted as M. The equilibrium between A^- , Na^+ and M is given by the following equation:

$$nA^- + mNa^+ \rightarrow M^{(n-m)^-} \qquad (1)$$

The value of "n-m" is the total number of free charge on the micelle and m is less than n. Eqilibrium constant can be found out from eqation 1 which is as follows:

$$K_m = \frac{[M]}{[A^-]^n[Na^+]^m} \qquad (2)$$

The free energy change is given by the equation:

$$\Delta G = \frac{RT \ln K_m}{n} \qquad (3)$$

Here R is the molar gas constant and T is the absolute temperature. On substituting eqation 1 in equation 2 and changing natural logarithm to $\log_{10}$ a relation was found as follows:

$$\frac{\Delta G}{2.303\,RT} = -\log\frac{[M]}{n} + \log[A]^{-} + \frac{m}{n}\log[Na]^{+} \qquad (4)$$

The bile salt concentration at equilibrium $[A^{-}]$ will be the CMC of the solution. If we assume the free energy change during micellisation to be constant in ionic detergents on addition of counter ions then the left side of equation 4 becomes a constant. Since the overall micellar concentration $[A^{-}]$ minus the CMC at the critical miceller concentration is very small so the micellar term $\log\frac{[m]}{[n]}$ is considered as negligible irrespective of any value of "n". Thus equation 4 becomes:

$$\frac{\Delta G}{2.303RT} = \log CMC + \frac{m}{n}\log[Na]^{+} \qquad (5)$$

This equation is similar to the standard equation for straight line where $\frac{m}{n}$ is the slope of $\log CMC$ and $\log NaCl$. As the concentration of NaCl increases so the CMC of a bile solution decreases. This is the effect of salt on micelle formation in aqueous solution of bile salt.

1.7. OBJECTIVE OF THE STUDY:

The application of bile salt as a vehicle for drug delivery is conducive due to two reasons 1) its micellisation property and 2) biocompatible nature. The study of the physiological environment revealed that all body fluids have salts in certain concentrations. So the objective of the present work is to investigate the effect of different salts on the micellisation of bile salts by using spectroscopic techniques. For this study pyrene has been chosen as the chromophore and two bile salts sodium deoxycholate (NaDC) and sodium cholate (NaC) have been used. The salts used are NaCl, KCl, KBr, $CaCl_2$ and $MgCl_2$.

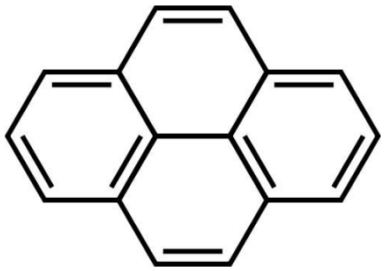
CHAPTER - 2

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Chromophore Used:

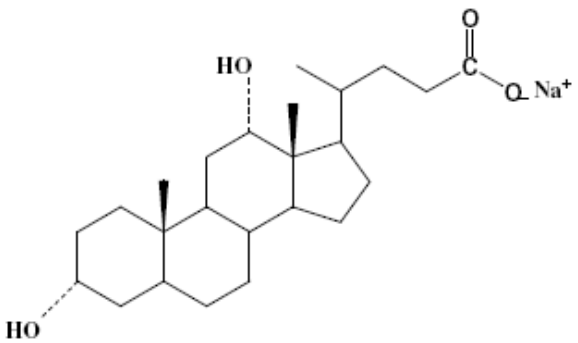
Pyrene was purchased from Sigma Chemical Company (USA). It was crystallised from ethanol and was used for the studies. .



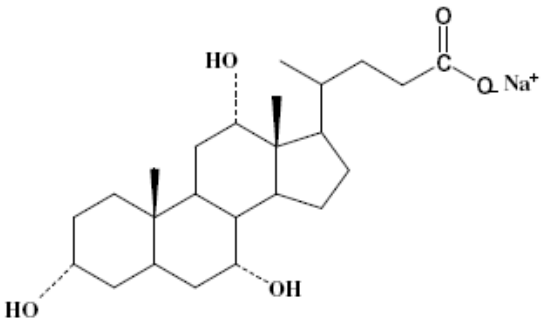
Molecular Structure of Pyrene

2.1.2 Medium Components:

The bile salts used were sodium cholate (NaC) and sodium deoxycholate (NaDC) bought from SRL Chemical Co. India.



Sodium Deoxycholate (NaDC)



Sodium Cholate (NaC)

The salts used were all of AR grade. The salts used were sodium chloride (NaCl), potassium chloride (KCl), potassium bromide(KBr), calcium chloride($CaCl_2$) and magnesium chloride ($MgCl_2$).

2.1.3 Solvents:

Deionised water was used for all the experiments. Methanol (SRL Chemical Co., India) of spectrograde quality was used to prepare the stock solution of pyrene.

2.1.4 Instrumentation:

The absorption spectra were recorded using ***Shimadzu Spectrophotometer (UV-2450).***

2.2 METHODS:

2.2.1 Preparation of Bile Salts Solution:

The stock solutions of NaC (32 mM) and NaDC (30 mM) were made in deionised water. Then on serial dilution the reqired concentrations of experimental solutions were obtained. The stock for the study of salt effect was made in the desired amount of salt solutions. Every time fresh solution of bile salt were made to avoid the problem of ageing.

2.2.2 Preparation of Probe Solution:

The stock solution (1 mM) of pyrene was made in methanol of spectroscopic grade. The experimental solutions were made by adding few microlitres of the methanolic solution of pyrene to the bile salt solution. The contamination of methanol in the experimental solution was maintained very low to avoid any perturbation to the micellisation process. Concentration of pyrene stock was maintained at $1x10^{-5}$ M for absorption studies.

2.3 TECHNIQUES USED:

2.3.1 Measurement of Absorption Spectrum:

The main elements of UV-VIS spectrophotometer are a light source, a monochromator and a detector. The monochromator works as a diffraction grating to dispense the beam of light into various wavelengths. The detectors role is to record the intensity of the light which has been transmitted. Before the samples are run, a reference must first be taken. This calibrates the spectra to screen out any spectral interference. In this case milipore water used as solvent to dissolve sample hence it was used as reference. The spectrum was recorded between 200 nm to 600 nm in a single fast scan.

CHAPTER - 3

RESULTS AND DISCUSSION

3.1 ABSORPTION STUDIES:

3.1.1 Study of Bile Salts in Water

Pyrene is one of the most widely used chromophore for the study of micellisation process of various surfactants. UV-Vis absorption studies were carried out to check the effect of bile salts on the absorption behavior of pyrene. Fig 3.1A and 3.1B represent the absorption spectra of pyrene with increasing concentrations of NaDC and NaC, respectively. The structured absorbance band in the wavelength range of 280 nm-350 nm is due to the pyrene monomer absorption. With increasing NaDC and NaC concentrations pyrene aggregates get disaggregated. The increased absorption of the pyrene monomer indicates that pyrene gets solubilised in the bile salt micelles at high concentration of bile salts.

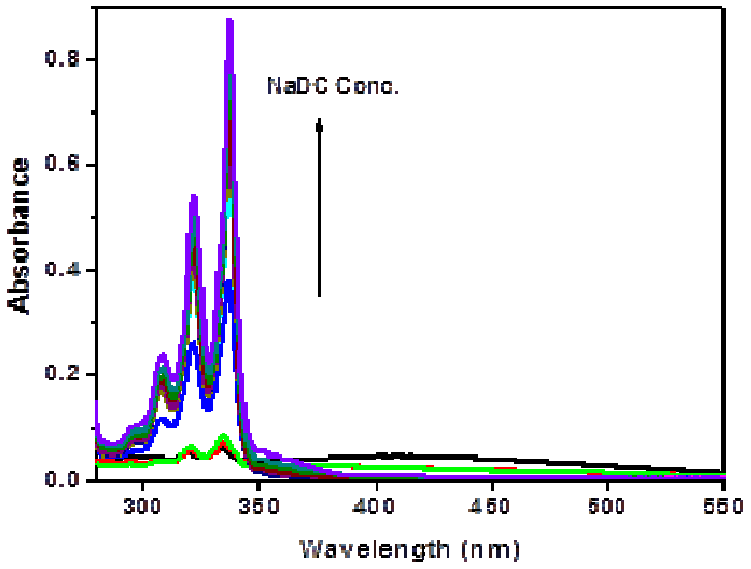


Fig. 3.1(A) Absorption spectra of pyrene in NaDC in water with increasing concentration of NaDC. Temperature = 25^0C, [pyrene] = 1.5 x 10^{-5} M, [NaDC] = 0-20 mM.

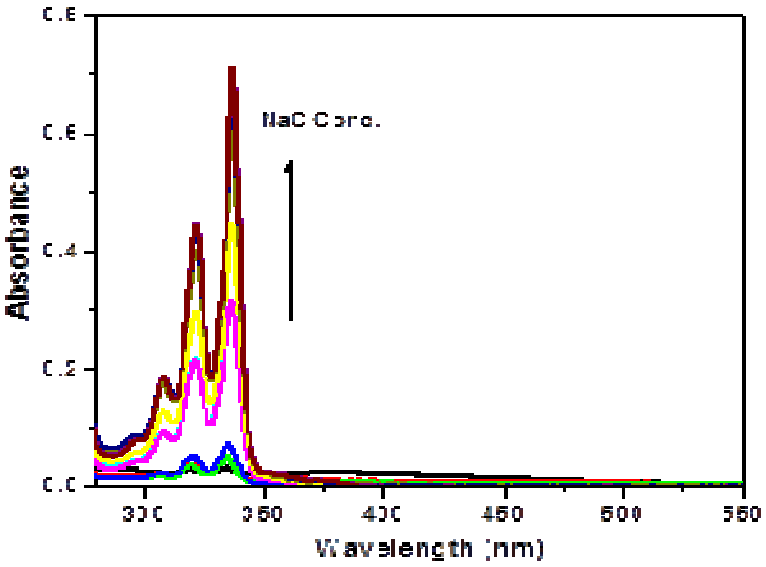


Fig. 3.1(B) Absorption spectra of pyrene in NaC in water with increasing concentration of NaC. Temperature = 25^0C, [pyrene] = 1.5 x 10^{-5} M, [NaC] = 0-32 mM.

3.1.2 Calculation of Critical Micellar Concentration (CMC):

The change in the absorbance value accompanying the transfer of pyrene molecules from a water environment to the micellar microenvironment can provide information about the critical micelle concentration (CMC) of bile salt. In order to calculate the CMC for NaDC and NaC, the absorbance value is plotted against the concentration of NaDC and NaC, respectively (Fig. 3.2A and 3.2B). The CMC values estimated from the plots are ≈ 6 mM for NaDC and ≈ 16 mM for NaC.

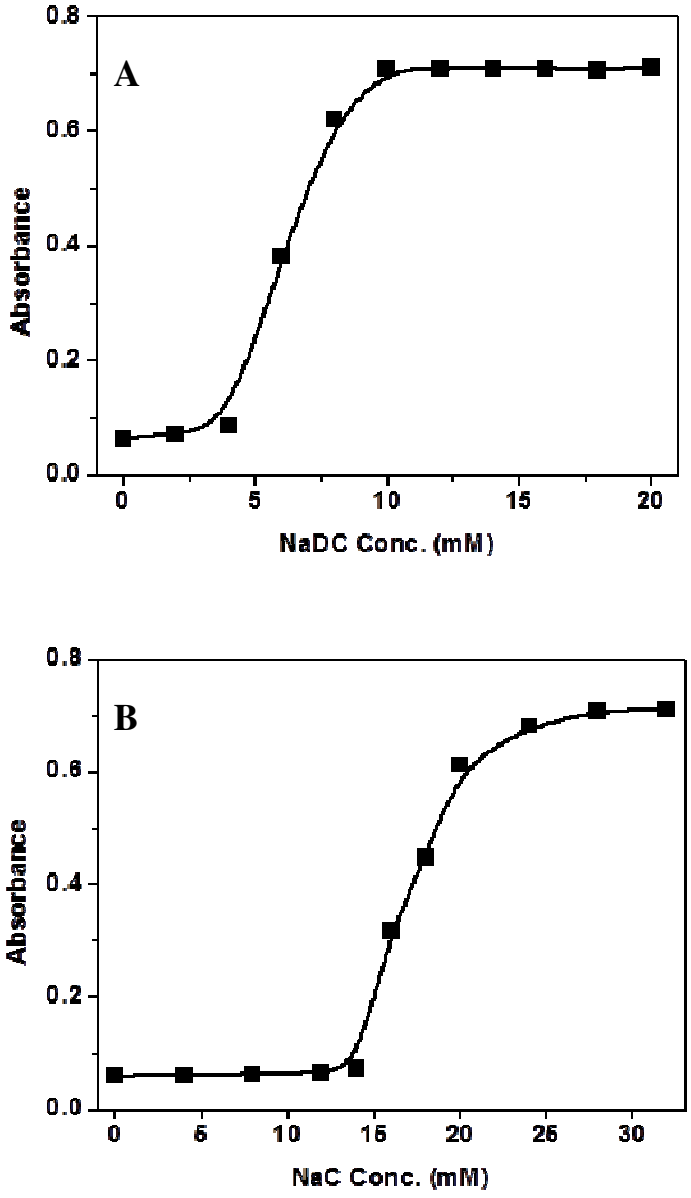


Fig. 3.2 Variation of absorbance of pyrene as a function of **(A)** NaDC and **(B)** NaC concentrations

3.2 EFFECT OF NaCl ON MICELLISATION OF BILE SALTS

3.2.1 UV-Vis Absorption Studies

In order to observe the effect of salts on the micellisation process, UV –Visible studies were carried out for NaDC and NaC in presence of various salts. In the present studies the salt concentrations were chosen as 0.05 M and 0.1 M.

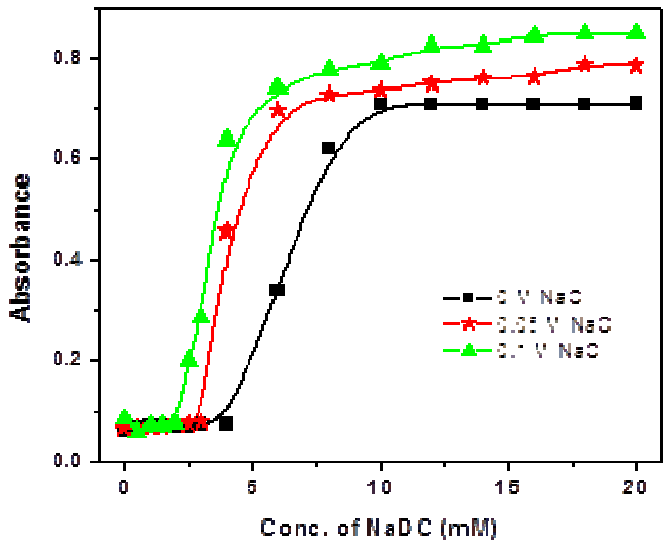


Fig. 3.3 Variation of absorbance of pyrene as a function of NaDC concentration in 0 M, 0.05 M, 0.1 M of NaCl.

Fig.3.3 shows the variation of absorbance of pyrene obtained from the changing concentration of NaDC in 0 M, 0.05M and 0.1M NaCl. In presence of salt there is a lowering of CMC value observed. The CMC of NaDC shifts from 6 mM to 3 mM in presence of 0.05 M NaCl and to 2 mM in presence of 0.1 M NaCl. Similar observation was obtained for NaC where the CMC got shifted from 16 mM in water to 12 mM in presence of 0.05 M NaCl and 10 mM in presence of 0.1 M NaCl.

The inference drawn from this observation is that the presence of NaCl facilitates the micelle formation at lower concentration of bile salts. Thus in the body fluid due to the presence of salt, bile salt micelles are formed at lower concentrations. Apart from NaCl there are several other cations (K^+, Ca^{+2}, Mg^{+2} *etc.*) present in the body fluid. Experiments were carried out to investigate the effect of these cations on the micellisation process of bile salts.

3.3 EFFECT OF KCl ON MICELLISATION OF BILE SALTS

In order to check the effect of potassium ion on the micellisation process experiments were carried out with KCl. By comparing the data for NaCl and KCl, the effect of potassium ion will be clear since both salts have the same counter anion (Cl^-). Similar to NaCl work 0.05 M and 0.1 M KCl were used to solubilise NaDC and NaC. The absorption spectral behavior was found to be very similar to that in case of NaCl. Fig.3.4 shows the variation of absorbance of pyrene obtained from the changing concentration of NaDC in 0.05 M and 0.1 M KCl.

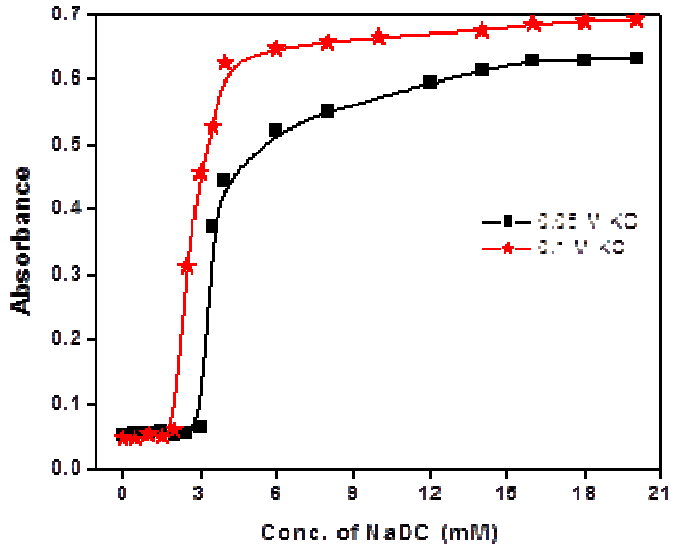


Fig. 3.4 Variation of absorbance of pyrene as a function of NaDC concentration in 0.05 and 0.1M KCl.

The CMC change is also same as recorded in 0.05 M and 0.1 M NaCl. The value changed from 3 mM in 0.05 M KCl to 2 mM in 0.1 M KCl. Since both NaCl and KCl have similar effect on the micellisation process it can be inferred that charge remaining the same change of cation does not have any effect on the micellisation process of bile salts. Now the question is whether the anion per se plays any significant role.

3.4 EFFECT OF KBr ON MICELLISATION OF BILE SALTS

In order to get an answer to the above question experiments were carried out in presence of 0.05 M and 0.1 M of KBr. The effect of KBr on the absorption behavior of pyrene in NaDC bile salts solutions was very similar to that of KCl and NaCl.

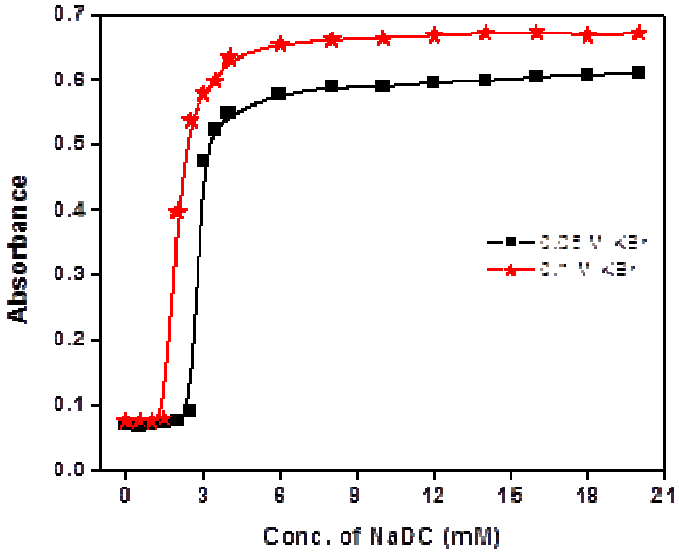


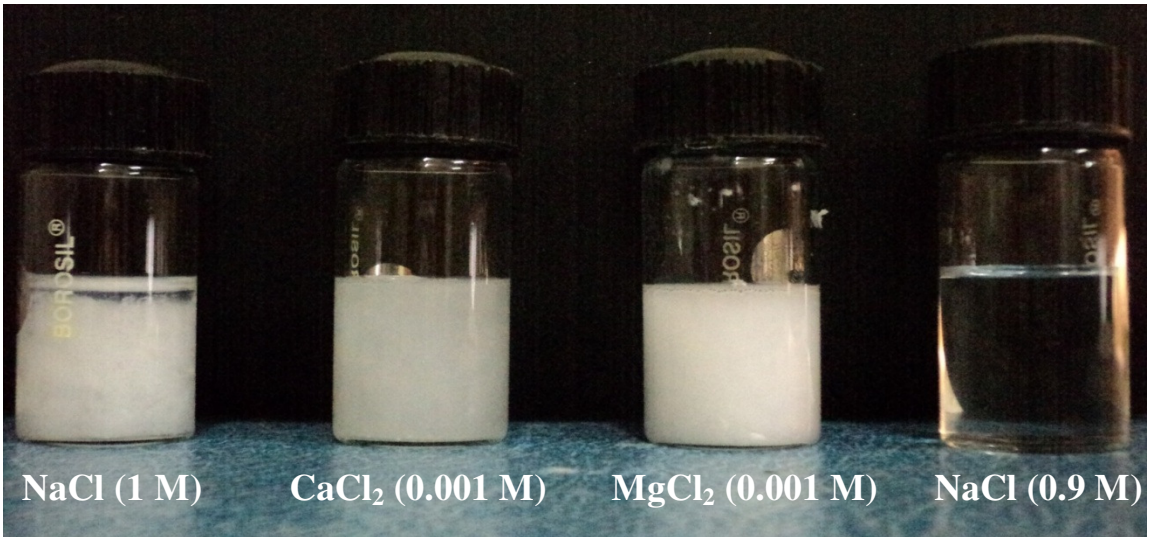
Fig. 3.5 Variation of absorbance of pyrene at 337 nm as a function of NaDC concentration in 0.05 and 0.1 M KBr.

Fig. 3.5 shows the variation of absorbance of pyrene obtained from the changing concentration of NaDC in 0.05M and 0.1M KBr. The CMCs calculated are also same *i.e.* 3 mM in 0.05 M KBr to 2 mM in 0.1 M KBr. Thus it can be concluded that by changing the anion from chloride to bromide does not have any effect in the micellisation behavior. So it is the charge on the cation and anion that matters and not the size.

3.5 EFFECT OF CHARGE OF THE CATION ON THE MICELLISATION PROCESS:

In order to test out the above proposition work was planned to carry out similar studies in presence of divalent alkaline earth metal salts $CaCl_2$ and $MgCl_2$. But to our surprise even at a very low concentration of these two salt solutions (0.001M) resulted in turbidity in the bile salt

stock solution. But for NaCl even upto a concentration of 0.9 M the bile salt solution was clear and turbidity appeared only in presence of more than 1 M of NaCl. The photograph from left to right shows the NaDC solution (20 mM) in presence of NaCl (1 M), $CaCl_2$ (0.001 M), $MgCl_2$ (0.001 M) and NaCl (0.9 M). Further investigations are under progress to understand the above observation.



Photograph of NaDC solution (20 mM) in aqueous medium containing NaCl (1 M), $CaCl_2$ (0.001 M), $MgCl_2$ (0.001 M) and NaCl (0.9 M) from left to right, respectively.

CONCLUSIONS:

- Effect of various salts (NaCl, KCl, KBr, $CaCl_2$ and $MgCl_2$) on the micellisation process of two bile salts NaDC and NaC was investigated.

- Pyrene was used as a chromophore for the study. The absorption and fluorescence properties of pyrene were used for probing the micellisation process.

- Presence of a small amount of NaCl (0.05 M) facilitates the micellisation process of both NaDC and NaC.

- CMC value decreases in presence of NaCl. For NaDC it drops from 6 mM at 0 M salt to 3 mM at 0.05 M salt and to 2 mM at 0.1 M salt. For NaC the drop is from 16 mM at 0 M salt to 12 mM at 0.05 M salt and to 10 mM at 0.1 M salt.

- KCl and KBr have similar effect on the micellisation process as that of NaCl.

- Even a small amount (0.001 M) of $CaCl_2$ or $MgCl_2$ induces turbidity in the bile salt solution.

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